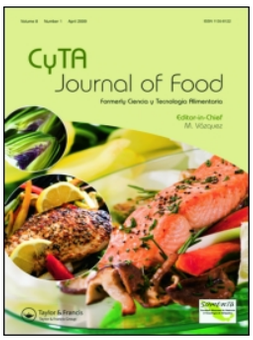

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Variations in fatty acid composition and antioxidant content in olive fruits as a result of the application of phytoregulators to the olive tree (*Olea europaea* L.)

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ABSTRACT

A new procedure to minimize the degradation of fatty acids and polyphenols during the olive storage is here proposed. This procedure is based on pre-harvest treatments of *Arbequina* and *Picual* olive trees with methyl jasmonate and abscisic acid. As a result, total phenolic content increased in all treated *Arbequina* olives as compared with controls. In contrast to *Arbequina*, only abscisic acid resulted in total phenolic content increase in *Picual* olives. Regarding the antioxidant activity, neither of the phytoregulators was effective. Fatty acid composition was also affected by both of them in *Arbequina* and *Picual*. Saturated fatty acid decreased with both methyl jasmonate and abscisic acid whereas unsaturated fatty acids increased significantly in treated olives. Olives with higher antioxidant activity exhibited higher unsaturated/saturated fat ratio. The application of phytoregulators to olive tree can be interesting to the oil industry particularly when a delay in oil processing occurs and olives have to be stored.

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Olive; methyl jasmonate; abscisic acid; fatty acids; polyphenols

PALABRAS CLAVE
aceituna; jasmonato de metilo; ácido abscísico; ácido grasos; polifenoles

Variaciones en la composición de ácidos grasos y contenido antioxidante en aceitunas como resultado de la aplicación de fitorreguladores al olivo (*Olea europaea* L.)

RESUMEN

En este trabajo se propone un nuevo procedimiento para minimizar la degradación de ácidos grasos y polifenoles durante el almacenamiento de la aceituna. Este procedimiento se basa en el tratamiento pre-cosecha de olivos *Arbequina* y *Picual* con jasmonato de metilo y ácido abscísico. Como resultado, el contenido de fenoles totales aumentó en todas las aceitunas *Arbequina* tratadas por comparación con las aceitunas control. Al contrario que en *Arbequina*, sólo el ácido abscísico provocó un aumento de fenoles totales en el caso de las aceitunas Picual. Con respecto a la actividad antioxidante, ninguno de los dos fitorreguladores resultó efectivo. La composición de ácidos grasos también se vio alterada con los tratamientos en *Arbequina* y en *Picual*. Los ácidos grasos saturados descendieron tanto con jasmonato de metilo como con ácidos abscísico mientras que los ácidos grasos insaturados aumentaron significativamente en las aceitunas tratadas. Las aceitunas con mayor actividad antioxidante mostraron una mayor relación entre ácidos grasos insaturados y ácidos grasos saturados. La aplicación de fitorreguladores al olivo puede ser interesante para la industria del aceite, especialmente cuando existen retrasos en el procesado y las aceitunas tienen que ser almacenadas.

1. Introduction

Both olive oil and table olives are widely popular in the Mediterranean area due to their pleasant taste and nutritional properties. The beneficial effects of olive oil and olive fruit are in part associated with the high content of monounsaturated fatty acids (MUFAs) in relation to polyunsaturated fatty acids (PUFAs) (Schwingshack & Hoffman, 2014). The main MUFA in olive is oleic acid, accounting up to 75% of the total lipidic composition. The other major fatty acids (FAs) present are the polyunsaturated linoleic acid (2.5-20%) and the saturated palmitic acid (10-20%) (Kiritsakis & Christie, 2000). Epidemiological studies reflect that a higher proportion of monounsaturated fats in the diet is linked with a reduction in the risk of cardiovascular diseases (Schwingshack & Hoffman, 2014). Reports have proven that the high-oleic content in olives is responsible for the olives´ ability to lower blood pressure and help control cholesterol (Georgakouli et al., 2016). Additional benefits of olive FAs are their capacity to decrease cardiovascular inflammation and protect arteries (Chiuve et al., 2012). Apart from the healthy properties of FAs, olives also contain some bioactive minor components. Among them, particular attention is paid to polyphenols because of their antioxidant characteristics (Casas, Estruch, & Sacanella, 2018). Polyphenols contribute not only to the health attributes of olives but also to the oxidative stability of olive FAs, mainly PUFAs.

The quality of olive oil depends on a number of factors (Ghabi et al., 2015). However, one of the most relevant factors is the delay in olive processing and, as a consequence, the need for fruit storage after harvesting

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(Spika, Olivera Koprivnjak, Skevin, Zanetic, & Katalinic, 2015). During the storage, compositional changes in olive have been described. In particular, polyphenol content decreases up to a third as a result of chemical degradation (Benito, Oria & Sánchez-Gimeno, 2009). To guarantee the olive fruit quality during the storage, good agricultural practices that preserve the antioxidant content are still sought.

In this context, the application of phytoregulators to plant foods has been lately described to enhance the content of bioactive components, antioxidants among others. Some of the most used phytoregulators are the phytohormones abscisic acid (ABA) and methyl jasmonate (MJ) (Huang et al., 2016). ABA is rapidly accumulated in response to stress whereas MJ acts as a triggering molecule to stimulate the production of secondary metabolites. The effectiveness of both ABA and MJ in inducing the formation of antioxidant compounds such as polyphenols has been largely reported (Blanch, Flores, Gómez-Jiménez, & Ruiz Del Castilo, 2018a, 2018b; Yang et al., 2012). Also, MJ's influence on FA composition has been occasionally studied in buckwheat seedlings (Klocek et al., 2016) and blackcurrant seeds (Flores & Ruiz Del Castillo, 2016).

In the present study, a new pre-harvest procedure based on the application of ABA and MJ to the olive tree (*Olea europaea* L.) is proposed to preserve the oxidative stability of olive fruits during the storage. To that end, total phenol content (TPC), free radical scavenging activity and FA content were determined in olive fruits harvested from the trees treated with ABA and MJ after 30-day storage. The results were compared with those of untreated olive fruits, which were used as a reference. The final purpose of this study was to develop an agricultural practice allowing FA oxidation to be minimized and hence oil quality to be guaranteed during olive fruit storage.

2. Materials and methods

2.1. Samples and chemicals

HPLC-grade MeOH and EtOH were supplied by VWR Inc. (Bridgeport, PA, USA). Ultrapure water was collected from a purification system (Millipore Milford, MA, USA). MJ was obtained from Sigma-Aldrich (Steinheim, Germany) and ABA was purchased from Across Organics (New Jersey, USA). Glacial acetic acid was obtained from Probus S.A. (Barcelona, Spain) and sodium chloride from Quimicen (Madrid, Spain). Sodium methoxide, 2,6-di-*tert*-butyl -4-methylphenol (BHT), 1,1-diphenyl-2-picrylhydrazil (DPPH) and the methyl esters of myristic (C14:0), palmitic (C16:0), stearic (C18:0), arachidic (C20:0), oleic (C18:1) and linoleic (C18:2) and linolenic acid (C18:3) were all purchased from Sigma-Aldrich (Milan, Italy). Sodium carbonate, Folin-Ciocalteu reagent were supplied by Merck (Darmstadt, Germany). Olive fruits (*Arbequina* and *Picual* cultivars) were hand-picked from the trees treated with the phytoregulators in November and December 2016 in the University of Extremadura (Badajoz, Spain).

2.2. Plant material and pre-harvest treatments

For our study, twenty-year-old olive trees (*Olea europaea* L.) from two different cultivars (*Arbequina* and *Picual*) in the same orchard near Badajoz (Spain) were used. They were grown under trip irrigation and fertirrigation, which is a type of irrigation including suitable fertilizers in the solution. All the olive trees were grown under the same agronomical and environmental conditions. The application of MJ and ABA was accomplished to olive trees (5 per treatment) by selecting two branches per tree with uniform size and fruit load. The treatments (a solution of 500 mg/L of MJ in ethanol and an aqueous solution of 100 mg/L of ABA) were applied during the *veraison* transition. For each treatment, five branches (one branch for a tree) were sprayed with the solution and the other five branches (one branch for a tree) were not sprayed to be used as controls. A 500 mL volume of each phytoregulator solution was applied per branch at the time of harvest. During the application of the treatment, at least one guard tree between test trees was used to avoid contamination. Besides, the spraying was only carried out on no windy days. Olive fruits of each cultivar were picked from each tree (300 fruits) on days 3 and 6 after applying the treatments. For the experiments, only intact fruits with the same maturity stage and similar appearance were selected. Then, the olives collected were split into two different batches for their analyses: one for total phenol content (TPC) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) activity, and the other for oil content and FFA composition. Olives were kept in cool bags and immediately subject to analysis as detailed below.

2.3. Total phenol content (TPC) and 2,2-diphenyl-1-picrylhydrazyl (DPPH)

2.3.1. Extraction

Prior to the actual analysis, the olives were de-stoned. The isolation of polyphenols was based on the procedure developed by Vinha et al. (2005). In brief, a 5 g weight of olive sample was mixed with a 60 mL volume of 80:20 (v/v) methanol:water. After that, the mixture was homogenized with an Ultraturrax (IKA, Sigma-Aldrich, Madrid, Spain) and centrifuged at 252 rfc for 10 min at room temperature. Subsequently, the supernatant was carefully taken and filtered. The extract was then extracted again with 60 mL of methanol:water. To avoid interferences, the remaining oil was removed with 30 mL of hexane. The hexane layer was discarded and the methanolic extracts were combined and filtered through Whatman No. 1 filter paper. Finally the clean extract was analyzed by HPLC as explained below. All extractions were performed in duplicate for each treatment.

2.3.2. Total phenol content (TPC)

The measurements were carried out by using a Beckman Coulter DU-800 spectrophotometer (Barcelona, Spain). The method used to determine TPC was that earlier described in the literature (Singleton & Rossi, 1965). This method is based on the use of the Folin-Ciocalteu reagent, which oxides the hydroxyl groups of phenols. In brief, 0.5 mL of Folin-Ciocalteu reagent and 10 mL of sodium carbonate solution (75 g/L) were added to a 0.1 mL volume of the extract. This mixture was made up to 25 mL with distilled water and was left for 1 h. Then the absorbance was measured at 750 nm and it was compared with that obtained from the blank, which was prepared equally to the sample but without adding the Folin-Ciocalteu reagent. Calibration curves were prepared by using gallic acid. The results obtained were expressed as milligrams of gallic acid equivalents

per kg of olive fruit. All analyses were accomplished in triplicate.

2.3.3. 1,1-Diphenyl-2-picrylhydrazyl free radical (DPPH*) scavenging assay

A Beckman Coulter DU-800 spectrophotometer (Barcelona, Spain) was used to measure the result of the DPPH assay. The method applied to determine the antioxidant activity of the extracts was developed by Smith, Reeves, Dage, and Schnettler (1987). Each extract was diluted to final concentrations of 15.6, 62.5, 125, 250 and 500 µg/mL and then they were transferred to a 96-well microliter plate. In each well, a 150 µL volume of a DPPH solution (400 µM) was added to 50 µL aliquot of the sample. The mixture reacted for 30 min at 37°C and the absorbance was measured at 517 nm. The value obtained was compared with that provided by the DPPH solution and each extract, without DPPH, was used as a blank. The percentage inhibition of the DPPH by each dilution of samples was estimated by considering the percentage of the steady DPPH in solution after reaction. The results were expressed as the concentration of extracts that gives rise to a 50% decrease in the DPPH. All the experiments were performed out in triplicate.

2.4. Oil content and FFA composition

2.4.1. Oil content

Olive fruits (5 g) were cleaned, de-stoned and dried in an oven at 80°C to constant weight. After that, 20 mL of (MeOH: CHCl3; 1:1) (v/v) was added to the dried and de-stoned olive samples and the mixture was transferred to a test tube. The mixture was then homogenized for 10 min using an Ultra-Turrax blender and extracted twice at 90°C for 2 h. The extracts were collected and the solvent was evaporated in a rotary evaporator at 35°C to dryness. Oil content was expressed as percentage of dry matter and the FFA composition was determined. The starting free fatty acid (FFA) composition was additionally determined on the treatment day (so-called day 0) to assess the natural evolution of FA acid composition with the time.

2.4.2. FFA derivatization

Prior to GC analysis, derivatization of the extracted FFAs was necessary. This way, fatty acid methyl esters (FAMEs) were formed by using a transesterification method previously developed, which described the derivatization and extraction in just one step (Ruiz Del Castillo, Dobson, Brennan, & Gordon, 2002). Basically, 2.0 ml of MeOH and 2.0 ml of 0.5 N sodium methoxide, which was used as a derivative reagent, were added to 100 mg of sample. The resulting mixture was then heated at 50°C for 10 min. After that, 3 ml of MeOH containing butylated hydroxytoluene (BHT; 50 mg/L), which was used as an antioxidant, 100 µl of glacial acetic acid and 5 ml of saturated sodium chloride were added. The mixture was shaken and centrifuged at 10 rfc for 5 min. Finally, the upper layer was eliminated and taken to dryness by putting through an anhydrous sodium sulfate column.

2.4.3. Analysis of FAMEs by GC

A gas chromatograph (Shimadzu model AOC-20i) equipped with a split/splitless injector system and a flame ionization detector (FID) was used to determine the extracted FFAs. The separation was carried out on a 25 m x 0.25 mm i.d. capillary column coated with a 0.25 µm layer of polyethylene glycol (007 Carbowax 20M, Quadrex). The carrier gas was helium and the flow rate was set at 1 ml/min. The temperatures of injector (in the splitless mode) and detector were 250°C and 320°C, respectively. The GC column was programmed at 4°C/min from 70°C to 190°C. Shimadzu MDGC solution system was used for data acquisition. Retention times of the chromatographic signals were compared with those provided by the FAME standards run under identical conditions. Total oil contents (wt % wt-1) were measured gravimetrically whereas FA composition was expressed as weight percents of total FAs.

2.5. Statistical analyses

Analysis of variance (ANOVA) of data on the influence of treatments with MJ and ABA on TP, DPPH and FFA composition was performed using JMP Statistics software package version 8 (purchased from SAS Institute Inc., North Carolina). The effect of the phytoregulators was assessed by the Fisher test. Differences between data were compared by least significant differences (LSD). The values used were always the mean of the three replicates performed. Differences at $p < .05$ were considered to be significant.

3. Results and discussion

3.1. Total phenol content (TPC) and 2,2-diphenyl-1-picrylhydrazyl (DPPH)

As can be seen in Figure 1, general TPC values in controls ranged from 127.7 to 472.2 mg gallic/kg which is in accordance with data published in olives in the literature (Arslan & Özcan, 2011; Flores, Blanch, & Ruiz Del Castillo, 2017). Besides, the TPCs in controls were significantly ($p < 0.05$) higher in *Picual* than in *Arbequina* (472.2 *vs* 265.6 mg gallic/kg on day 3 and 338.3 *vs* 155.9 mg gallic/kg on day 6). Varietal differences in olive TPC have been previously described in the literature (Petridis, Therios, & Samouris, 2012). In addition, TPC also varied according to the picking day within each cultivar being higher on day 3 for both cultivars. Decreases in TPCs as ripening progress have been reported in the literature (Cevik, Ozkan, Kiralan, & Bayrak, 2014). Therefore, the lower TPC values measured on day 6 probably indicate that olives picked on day 6 were closer to overripe than those picked on day 3 for both *Arbequina* and *Picual*.

Interestingly, the pre-harvest MJ treatment effect was cultivar dependent. For *Arbequina*, MJ treatment resulted in a significant ($p < 0.05$) increase in TPC as compared with controls (430.5 *vs* 265.6 mg gallic/kg on day 3 and 434.2 *vs* 155.9 mg gallic/kg on day 6, respectively). On the contrary, TPC decreased significantly ($p < 0.05$) after MJ treatment for *Picual* on both 3 and 6 picking days (380.1 *vs* 472.2 mg gallic/kg on day 3 and 127.7 *vs* 338.3 mg gallic/kg on day 6).

Regarding the DPPH activity, it is worth pointing out that no significant ($p < 0.05$) variation was observed in controls. Therefore, there was no direct relation between TPC and DPPH activity. This reflects the presence in some extracts of major compounds whose contribution to the free radical scavenging activity is little or null. For instance, *Picual* controls picked on day 3 exhibited higher TPC than the rest of

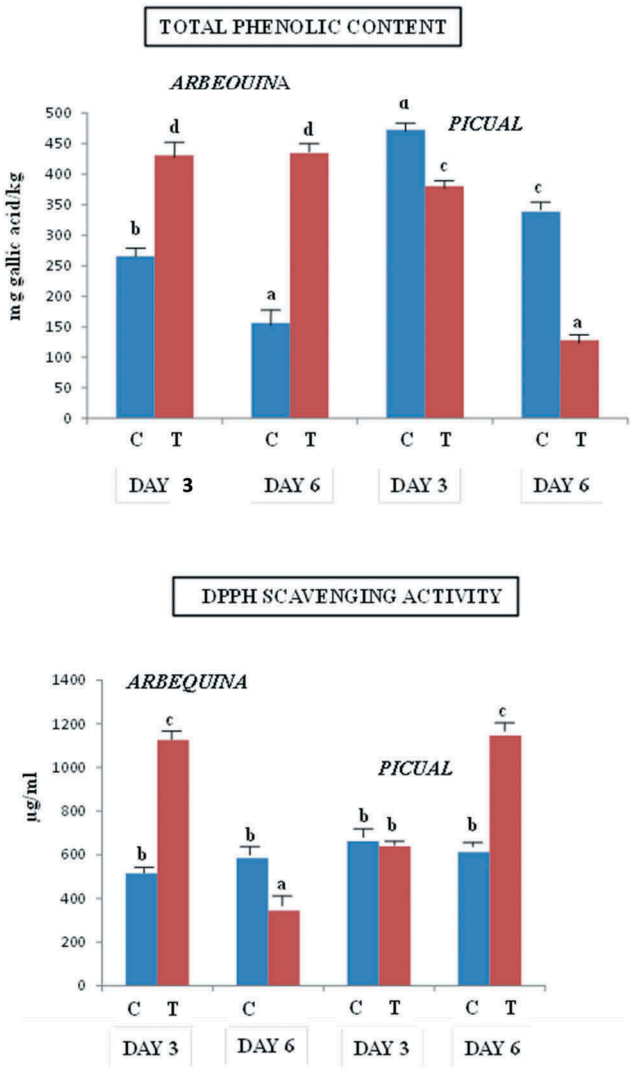

Figure 1. Total phenolic content (expressed as mg gallic acid/kg fresh fruit) and free radical scavenging activity (expressed as IC50, concentration of extract that gives rise to a 50% reduction in the DPPH), of olive fruits untreated-control (indicated as C) and treated with MJ (indicated as T) on days 3 and 6 after treatment. Data on *Arbequina* and *Picual* cultivars are included. The values were estimated as means ± SD (n = 3). Different letters indicate significant ($p < 0.05$) differences between control and treated samples.

Figura 1. Contenido en fenoles totales (expresados como ácido gálico/kg de fruta fresca) y actividad de barrido de radicales libres (expresado como IC50), concentración de extracto que da lugar al 50% de la reducción del DPPH), de aceitunas control (indicadas como C) y tratadas con JM (indicadas como T) en los días 3 y 6 después del tratamiento. Se incluyen datos de los cultivos Arbequina y Picual. Los valores se estimaron como media ± SD (n = 3). Letras diferentes indican diferencias significativas ($p < 0.05$) entre muestras control y muestras tratadas.

controls (i.e., 473.2 mg gallic/kg) and, however its free radical scavenging activity did not differ significantly ($p < 0.05$) from the rest of the controls (i.e., 539.1 µg/ml). The pre-harvest MJ treatment brought about significant ($p < 0.05$) decrease in the antioxidant activity in *Arbequina* olives picked on day 3 (IC50 increased from 514.4 mg gallic/kg in controls to 1125.5 mg gallic/kg in treated) and *Picual* olives picked on day 6 (IC50 increased from 612.0 mg gallic/kg in controls to 1146.5 mg gallic/kg in treated). Only *Arbequina* olives picked on day 6 exhibited an increase in antioxidant activity after MJ treatment. (IC50 decreased from 585.0 mg gallic/kg in controls to 343.7 mg gallic/kg in treated).

Therefore, pre-harvest MJ treatment resulted in general in higher TPC and DPPH activity in *Arbequina* whereas lower TPC levels and DPPH activity were measured in *Picual* olives. These results suggest different behaviors in maturity of both cultivars and, as a consequence, *Picual* olives were picked closer to the overripe stage whereas *Arbequina* samples were fully ripened. MJ has been already reported to activate the enzymes involved in the biosynthesis of polyphenols (Asghari & Hasanlooe, 2016; Flores, de la Peña Moreno, Blanch, & Ruiz Del Castillo, 2014).

As seen in Figure 2, results on ABA treatment showed that TPC levels in controls for both cultivars and both picking days were now rather similar. In contrast, the DPPH activity varied significantly ($p < 0.05$) with both the cultivar and picking day. This confirms the compositional differences among the extracts above mentioned. Besides, ABA pre-harvest treatment triggered mostly a significant ($p < 0.05$)

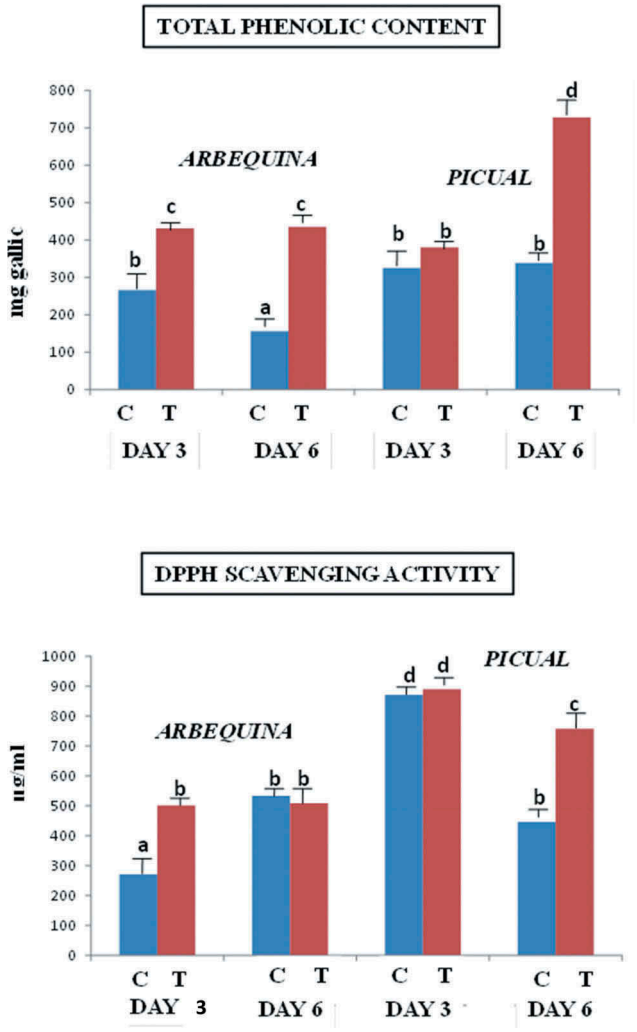

Figure 2. Total phenolic content (expressed as mg gallic acid/kg fresh fruit) and free radical scavenging radical (expressed as IC50, concentration of extract that gives rise to a 50% reduction in the DPPH), of olive fruits untreated-control (indicated as C) and treated with ABA (indicated as T) on days 3 and 6 after treatment. Data on *Arbequina* and *Picual* cultivars are included. The values were estimated as means ± SD (n = 3). Different letters indicate significant ($p< 0.05$) differences between control and treated samples.

Figura 2. Contenido en fenoles totales (expresados como ácido gálico/kg de fruta fresca) y actividad de barrido de radicales libres (expresado como IC50), concentración de extracto que da lugar al 50% de la reducción del DPPH), de aceitunas control (indicadas como C) y tratadas con ABA (indicadas como T) en los días 3 y 6 después del tratamiento. Se incluyen datos de los cultivos Arbequina y Picual. Los valores se estimaron como media ± SD (n = 3). Letras diferentes indican diferencias significativas ($p < 0.05$) entre muestras control y muestras tratadas.

increase of TPC, for both *Arbequina* and *Picual* olives on both 3 and 6 picking days. Regarding the antioxidant activity, the DPPH was inversely proportional to TPC values. This effect was particularly noticeable for *Arbequina* olives on day 3 (IC50 increased from 271.4 mg gallic/kg to 501.2 mg gallic/kg) and *Picual* olives on day 6 (IC50 increased from 445.5 mg gallic/kg to 757.7 mg gallic/kg). Therefore, ABA pre-harvest treatment resulted in an increase of the free radical scavenging activity on *Arbequina* olives on day 3 and *Picual* olives on day 6.

By comparing the effect of MJ and ABA, both of them increased TPC in *Arbequina* olives whereas only ABA was able to stimulate the accumulation of TP in *Picual*. In addition, TPC values reached with ABA were higher than those obtained from MJ treatment. Likewise, the study of the IC50 indicates that both elicitors caused a decrease of the free radical scavenging activity of olive fruits regardless the cultivar. An exception was found for *Arbequina* olives picked on day 6, whose DPPH activity increased after the exposition of olive trees to MJ. Additionally, IC50 values obtained from MJ were higher than those measured from ABA treated olives. Therefore, ABA resulted again more satisfactory than MJ in terms of DPPH activity. On the basis of the results on antioxidant activity, we elected *Arbequina* olives picked on day 6 and *Picual* olives picked on day 3 to evaluate the FA composition.

3.2. Oil and total fatty acid (TFA) contents

To estimate TFA depicted in Table 1, only the most important FAs (i.e., myristic palmitic, stearic, arachidic, oleic, linoleic, linolenic and stearidonic acids) were taken into account. Although some other minor peaks were also detected, their identification was uncertain and they were considered negligible. As seen, the oil content of *Arbequina* and *Picual* control olive fruits ranged from 45.3% to 49.0%. This oil content was very close to TFA content, which varied between 40.9% and 45.9%. This indicates that the oil content is approximately equal to TFA with the addition of small amounts of glycerol and non-saponifiable matter. These values are in the same range as those previously reported for olive fruits (Zarrouk et al., 2009). As also seen in Table 1, neither MJ nor ABA had significant ($p < 0.05$) effect on oil and TFA contents whatever the cultivar. It is likely that the treatments affect differently individual FAs in such a way that they are balanced in the total content.

Table 2. Fatty acid compositions (%) of untreated-control olive fruits picked from *Arbequina* and *Picual* trees on the harvest day.

Tabla 2. Composición de ácidos grasosa (%) de frutas sin tratar (control) recogidas de árboles *Arbequina* y *Picual* en el día de la cosecha.

	Composition (%)[a]	
Fatty acid	Arbequina	Picual
C14:0	0.60	0.42
C16:0	9.35	8.54
C18:0	11.92	11.09
C18:1	64.84	68.23
C18:2	8.88	8.57
C20:0	1.87	1.93
C18:3	2.54	1.22
ΣSFA[b]	23.74	21.98
ΣMUFA[c]	64.84	68.23
ΣPUFA[d]	11.42	9.79
U/S fat ratio[e]	3.2:1	3.5:1

[a]Values (% of total fatty acids) are means (n = 3); [b]SFA, saturated fatty acids; [c]MUFA, monounsaturated fatty acids; [d]PUFA, polyunsaturated fatty acids; [e]U:S, unsaturated:saturated ratio.

[a]Valores (% ácidos grasos totales) como media (n = 3); [b]SFA, ácidos grasos saturados; [c]MUFA, ácidos grasos monoinsaturados; [d]PUFA, ácidos grasos poliinsaturados; [e]Relación ácidos grasos insaturados/ácidos grasos saturados.

As seen in Table 2, the FA composition was the same in both cultivars. Olives were particularly rich in oleic acid which represented 64.84% in *Arbequina* and 68.23% in *Picual*. Oleic acid was therefore the major FFA component followed by stearic acid. Control olives on day 0 were also rich in SFA (i.e., 23.74% for *Arbequina*and 21.98% for *Picual*), which is mainly because of stearic and palmitic acids since myristic and arachidic acids are relatively low. PUFAs, composed of the sum of linoleic and linolenic acids, represented 11.42% in *Arbequina* and 9.79% in *Picual*. This composition agrees with bibliographic data (Kiritsakis & Christie, 2000). Also, U:S fat ratio, which is frequently used as a quality index, was 3.2:1 in *Arbequina* and 3.5:1 in *Picual*. This value is within the range regarded as optimum (Tartrakoon, Tartrakoon, & Kitsupee, 2016). From Table 3, the FA composition in controls was slightly different from that measured for the same samples on the harvesting day (Table 3 *vs* Table 2). SFAs in general increased after 30-day storage. In particular, ΣSFA enhanced from 23.74% on the harvesting day to 41.1% after 30-day storage in *Arbequina* and from 21.98% to 36.1% in *Picual*. In contrast, MUFAs and PUFAs decreased in control samples after the storage with respect to the harvesting day. Specifically, ΣMUFA dropped from 64.84% to 52.6% in *Arbequina* and from 64.23% to 58.9% in *Picual* whereas ΣPUFA decreased from 11.42% to 6.1% in *Arbequina* and

Table 1. Oil and total fatty acid (TFA) contents (% fruit dry weight basis) in olives untreated (control) and treated with methyl jasmonate (MJ) and abscisic acid (ABA). Data correspond to *Arbequina* olives picked on day 6 after treatment and *Picual* olives picked on day 3 after treatment and then stored for 30 days.

Tabla 1. Contenido de aceite y ácidos grasos totales (AGT) (% de fruta en base al peso seco) en aceitunas sin tratar (control) y tratadas con jasmonato de metilo (JM) y ácidos abscísico (ABA). Los datos se corresponden a aceitunas *Arbequina* recogidas el día 6 después del tratamiento y aceitunas *Picual* recogidas el día 3 después del tratamiento y luego almacenadas durante 30 días.

	Cultivars							
	Arbequina				*Picual*			
	Methyl jasmonate		Abscisic acid		Methyl jasmonate		Abscisic acid	
Olive content	*Control*	*Treated*	*Control*	*Treated*	*Control*	*Treated*	*Control*	*Treated*
OIL	45.3 ± 0.5a	42.8 ± 0.7a	46.1 ± 0.2a	44.4 ± 0.3a	48.7 ± 0.7a	46.5 ± 0.4a	49.0 ± 0.4a	50.1 ± 0.2a
TFA	41.1 ± 0.4a	40.5 ± 0.3a	40.9 ± 0.5a	41.8 ± 0.3a	45.2 ± 0.4a	44.8 ± 0.3a	45.9 ± 0.3a	43.6 ± 0.3a

Different letters in the same row indicate significant ($p < 0.05$) differences between control and treated samples.

Letras diferentes en la misma línea indican diferencias significativas ($p < 0.05$) entre muestras control y tratadas.

from 9.79% to 8.7% in *Picual* (compare Tables 2 and 3). This compositional variation is due to chemical degradation because of the storage, which is more pronounced in UFAs due to their susceptibility to oxidation.

By comparing MJ treated olives with those untreated, contents of SFA myristic, palmitic and arachidic acids dropped significantly ($p < 0.05$) after treatment. In particular, total SFA decreased from 41.1% in controls to 25.2% in *Arbequina*and from 36.1% in controls to 8.4% in *Picual*. Individual SFA also decreased after the exposition of olive trees to MJ. On the contrary, contents of UFAs oleic, linoleic and linolenic acids exhibited significant ($p < 0.05$) enhancement. The increase of oleic acid after MJ treatment was particularly noticeable. Similarly, linolenic acid increased significantly ($p < 0.05$) in treated samples. In fact, it could not be even detected in *Arbequina* controls whereas 6.1% was measured in MJ treated. MJ also resulted in an increase of linolenic acid in *Picual*. From Table 3, U:S fat ratio was also significantly ($p < 0.05$) higher as a result of MJ application. Particularly, U:S fat ratio increased from 1.5:1 to 4.4:1 in *Arbequina* and to 4.8:1 in *Picual*. Therefore, a better U:S fat ratio was also obtained after MJ pre-harvest treatment. MJ affects the enzymes responsible for the bioformation of FA. In this regard, the drop of SFA contents in treated samples reflects the inhibitory effect of MJ on the enzymes regulating the synthesis of *n*-16:0 and *n*-18:0, which is the first step in the biochemical pathway. These enzymes are packaged together in a complex called as an FA synthase (FAS). Elongases and desaturases are additional enzymes regulating the bioformation of other longer and shorter SFA further in the pathway. The significant ($p < 0.05$) increase of oleic acid after the application of MJ is indicative of the MJ promoting effect on stearoyl-ACP Λ9-desaturase activity, which is directly involved in the synthesis of oleic acid (Conde, Delrot, & Gerós, 2008). Regarding linoleic acid, it is likely that MJ is activating oleate desaturase enzyme. However, it is also possible that MJ is inhibiting LOX activity in such a way that oxidation of linoleic and linolenic acids would be minimized with the treatment. Actually, inhibitory effect of MJ on LOX enzyme was already proved in our laboratory in potato tubers in the past (Ruiz Del Castillo, Flores, & Blanch, 2010).

As seen in Table 4, the FA composition in controls in the ABA experiment was similar to that observed for controls in MJ experiment (Table 3). Oleic acid was by far the major FA (i.e., 50.3% and 50.8% in *Arbequina* and *Picual* respectively). Besides, the FA composition was also altered as a consequence of the storage (compare with data in Table 2). It is therefore confirmed the oxidative degradation of UFAs in control olives after 30-day storage.

From Table 4, SFA contents in ABA treated samples also decreased significantly ($p < 0.05$) as compared with controls. In particular, total SFA decreased from 40.6% to 18.4% in *Arbequina* and from 40.5% to 17.1% in *Picual*. Concerning individual SFA, all of them decreased significantly ($p < 0.05$) from controls to ABA treated, except stearic acid. In contrast to total SFAs, total PUFA increased significantly ($p < 0.05$) with ABA treatment, from 9.1% to 18.3% in *Arbequina* and from 8.7% to 18.4% in *Picual*. ABA effect on individual PUFA varied according to the specific compound. Whereas linolenic acid increased significantly ($p < 0.05$) with ABA in both cultivars (i.e., from 2.1% to 8.5% in *Arbequina* and from 1.5% to 9.3% in *Picual*), linoleic acid was not affected by the pre-harvest treatment. As seen in Table 4, it is also seen that oleic acid increased significantly ($p < 0.05$) after the exposition of olive trees to ABA. Finally, ABA effect on U:S fat ratio was the same as that observed for MJ. Both elicitors MJ and ABA resulted in a significant ($p < 0.05$) increase of U:S ratio, which is directly related to olive higher quality.

In view of these results, a relationship was established between the antioxidant activity (see Figure 1) and FA composition (see Tables 3 and 4). The higher the free radical scavenging activity of the extracts, the higher the U:S fat ratio. This indicates higher proportion of UFAs and lower

Table 3. Fatty acid composition (weight %) in untreated and pre-harvest methyl jasmonate (MJ) treated olive fruits. Two different cultivars, *Arbequina* and *Picual*, are included. Data correspond to *Arbequina* olives picked on day 6 after treatment and *Picual* olives picked on day 3 after treatment and then both stored for 30 days. Data on control samples after storage are also included for comparison.

Tabla 3. Composición de ácidos grasos (% en peso) en aceitunas sin tratar y tratadas con jasmonato de metilo (JM). Se incluyen dos diferentes cultivos, *Arbequina* y *Picual*. Los datos corresponden a aceitunas Arbequina recogidas en el día 6 después del tratamiento y a aceitunas Picual recogidas en el día 3 después del tratamiento y ambas almacenadas durante 30 días. Se incluyen también los datos de las muestras control después del almacenamiento para su comparación.

	Olive fruits[a]			
	Arbequina		*Picual*	
Fatty acids	Control	MJ Treated	Control	MJ Treated
C14:0	9.2 ± 0.2a	4.3 ± 0.1b	n.d.a	n.d.a
C16:0	17.7 ± 0.3a	11.3 ± 0.2b	19.1 ± 0.2a	1.9 ± 0.2b
C18:0	9.1 ± 0.1a	6.2 ± 0.2b	9.0 ± 0.1a	3.1 ± 0.1b
C18:1	52.6 ± 0.1a	64.6 ± 0.1b	58.9 ± 0.1a	68.5 ± 0.1b
C18:2	6.1 ± 0.1a	12.1 ± 0.2b	3.5 ± 0.3a	13.4 ± 0.1b
C20:0	5.3 ± 0.2a	3.4 ± 0.3a	8.0 ± 0.2a	3.4 ± 0.3b
C18:3	n.d.a	6.1 ± 0.2b	1.5 ± 0.3a	9.7 ± 0.4b
ΣSFA[b]	41.1 ± 0.3a	25.2 ± 0.3b	36.1 ± 0.2a	8.4 ± 0.2b
ΣMUFA[c]	52.6 ± 0.4a	64.6 ± 0.1b	58.9 ± 0.3a	68.5 ± 0.3b
ΣPUFA[d]	6.1 ± 0.1a	18.3 ± 0.1b	8.7 ± 0.1a	18.4 ± 0.1b
U/S fat ratio[e]	1.5:1a	4.4:1b	1.5:1a	4.8:1b

[a]Values (% of total fatty acids) are means ± SD (n = 3); [b]SFA, saturated fatty acids; [c]MUFA, monounsaturated fatty acids; [d]PUFA, polyunsaturated fatty acids; [e]U:S, unsaturated:saturated.

[a]Valores (% ácidos grasos totales) como media (n = 3); [b]SFA, ácidos grasos saturados; [c]MUFA, ácidos grasos monoinsaturados; [d]PUFA, ácidos grasos poliinsaturados; [e]Relación ácidos grasos insaturados/ácidos grasos saturados.

Different letters in the same row between control and treated samples within the same cultivar indicates significant changes at $p < 0.05$ level. Statistical comparison between *Arbequina* and *Picual* data was not carried out.

Letras diferentes en la misma línea entre muestras control y tratadas dentro del mismo cultivo indica cambios significativos a nivel $p < 0.05$. Comparación estadística entre los datos de *Arbequina* y *Picual* no se llevó a cabo.

Table 4. Fatty acid composition (weight %) in untreated and pre-harvest abscisic acid (ABA) treated olive fruits. Two different cultivars, *Arbequina* and *Picual*, are included. Data correspond to *Arbequina* and olives picked on day 6 after treatment and *Picual* olives picked on day 3 after treatment and then both stored for 30 days. Data on control samples after storage are also included for comparison.

Tabla 4. Composición de ácidos grasos (% en peso) en aceitunas sin tratar y tratadas con ácido abscísico (ABA). Se incluyen dos diferentes cultivos, Arbequina y Picual. Los datos corresponden a aceitunas Arbequina recogidas en el día 6 después del tratamiento y a aceitunas Picual recogidas en el día 3 después del tratamiento y ambas almacenadas durante 30 días. Se incluyen también los datos de las muestras control después del almacenamiento para su comparación.

	Olive fruits[a]			
	Arbequina		*Picual*	
Fatty acids	*Control*	*ABA treated*	*control*	*ABA treated*
C14:0	13.0 ± 0.1a	5.6 ± 0.2b	14.0 ± 0.1a	4.0 ± 0.1b
C16:0	17.5 ± 0.2a	9.1 ± 0.4b	16.9 ± 0.1a	9.4 ± 0.1b
C18:0	5.0 ± 0.2a	2.2 ± 0.1a	4.7 ± 0.2a	2.5 ± 0.2a
C18:1	50.3 ± 0.3a	63.3 ± 0.1b	50.8 ± 0.3a	64.5 ± 0.3b
C18:2	7.0 ± 0.1a	9.8 ± 0.1a	7.2 ± 0.2a	9.1 ± 0.2a
C20:0	5.1 ± 0.1a	1.5 ± 0.2b	4.9 ± 0.3a	1.2 ± 0.3b
C18:3	2.1 ± 0.2a	8.5 ± 0.3b	1.5 ± 0.3a	9.3 ± 0.1b
ΣSFA[b]	40.6 ± 0.3a	18.4 ± 0.4b	40.5 ± 0.4a	17.1 ± 0.2b
ΣMUFA[c]	50.3 ± 0.2a	63.3 ± 0.4b	50.8 ± 0.1a	64.5 ± 0.3b
ΣPUFA[d]	9.1 ± 0.2a	18.3 ± 0.3b	8.7 ± 0.1a	18.4 ± 0.1b
U/S fat ratio[e]	1.5:1a	4.4:1b	1.5:1a	4.8:1b

[a]Values (% of total fatty acids) are means ± SD (n = 3); [b]SFA, saturated fatty acids; [c]MUFA, monounsaturated fatty acids; [d]PUFA, polyunsaturated fatty acids; [e]U:S, unsaturated:saturated.

[a]Valores (% ácidos grasos totales) como media (n = 3); [b]SFA, ácidos grasos saturados; [c]MUFA, ácidos grasos monoinsaturados; [d]PUFA, ácidos grasos poliinsaturados; [e]Relación ácidos grasos insaturados/ácidos grasos saturados.

Different letters in the same row between control and treated samples within the same cultivar indicates significant changes at $p < 0.05$ level. Statistical comparison between *Arbequina* and *Picual* data was not carried out.

Letras diferentes en la misma línea entre muestras control y tratadas dentro del mismo cultivo indica cambios significativos a nivel $p < 0.05$. Comparación estadística entre los datos de *Arbequina* y *Picual* no se llevó a cabo.

proportion of SFAs. Therefore, the higher antioxidant content in the extracts contributes to higher oxidative stability of mainly PUFAs but also MUFAs. Several studies have shown that a diet rich in UFAs may result in a wide range of health benefits such as an improvement in cholesterol levels and, in turn, prevention of cardiovascular disorders (Aktas, 2013).

As a conclusion, when olive trees were treated with MJ or ABA, oxidation of FA in olive fruits was minimized and, therefore, the FA composition related with healthier attributes was observed. In this regard, high oleic acid content is correlated with lower risk of heart disease. In this line, high linoleic and linolenic acids are beneficial to maintain insulin and sugar levels in blood. In short, potential health promoting properties, particularly those related with HDL and LDL cholesterol in the blood are attributed to high U:S fat ratios (Milicevic et al., 2014). From a nutritional standpoint, pre-harvest MJ or ABA treated olives represent an interesting alternative to consume foods rich in UFAs. All in all, the results found in the present work demonstrate that the application of MJ or ABA to olive tree is a promising agricultural practice to guarantee the oil quality, particularly, when olives need to be stored.

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No financial interest or benefit that has arisen from the direct applications of our research so far. There is not any conflict of interest.

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